Photosynthetic Characteristics of Three Strains of Cyanobacteria Grown under Low- or High-CO₂ Conditions

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Abbreviations and Symbols:

CA: carbonic anhydrase
DCMU: 3-(3,4-dichlorophenyl)-1,1-dimethylurea
DIC: dissolved inorganic carbon
PS I; PS II: photosystem I or II
Fₚ: initial fluorescence
Fₘₐₓ: maximum fluorescence
Fᵥₐʳ: variable fluorescence
t₁/₂: half rise time of fluorescence
F₆₈₀₋₇₀₀: peak value between 680 and 700 nm
F₇₁₀₋₇₄₀: peak value between 710 and 740 nm
F₇₄₀₋₇₇₀: fluorescence at 77 K.

Introduction

Photosynthetic organisms change their affinities for external inorganic carbon depending on the concentration of CO₂ in the growth medium (Berry et al., 1976, Hogetsu and Miyachi, 1977). The activity of carbonic anhydrase (CA) and the accumulation of dissolved inorganic carbon (DIC) within their interior are much higher in algal cells which had been grown in air (containing about 0.04% CO₂; low-CO₂ cells) than in those which had been grown in air enriched with 1–5% CO₂ (high-CO₂ cells; cf. reviews of Raven, 1985; Aizawa and Miyachi, 1986). Photosynthesis is required for the accumulation of DIC in the cells (Spalding and Ogren, 1982; Kaplan et al., 1982). With low-CO₂ cells of Anabaena variabilis and Anacystis nidulans Ogawa et al. (1984, 1985) found that the photosystem I mediated cyclic electron flow is necessary for the accumulation of DIC. As a possible explanation for this effect it was discussed that an active transport system for DIC is driven by ATP produced by cyclic electron flow. A higher activity of PS I could be reflected in a higher quantum requirement and a higher ratio of F₇₁₀₋₇₄₀/F₆₈₀₋₇₀₀ fluorescence at 77 K.

Recently, we studied the effect of CO₂ concentration on quantum requirement of photosynthetic oxygen evolution and fluorescence emission spectra at liquid nitrogen temperature during growth of various species of unicellular green algae (Bürger et al., 1988). Three types of reactions were found. Firstly, in low-CO₂ cells of Dunaliella tertiolecta, Chlamydomonas reinhardtii C9 and Chlorella vulgaris 11g, both the quantum require-
ment and the ratio of $F_{710-740}/F_{680-700}$ fluorescence were higher in low-CO$_2$ cells than in high-CO$_2$ cells, indicating an uneven distribution of excitation energy between the photosystems with an enhanced excitation of PS I. Similar results were obtained for *Chlamydomonas reinhardtii* by Palmqvist et al. (1990). Upon transfer from high- to low-CO$_2$ condition the ratio of PS II/PS I activity decreases. This is discussed to be the result of a protein-phosphorylation of the light harvesting complex and a subsequent state 1 to state 2 transition.

Secondly, in *Chlorella pyrenoidosa*, although the quantum requirement for low-CO$_2$ cells was higher than in high-CO$_2$ cells, we found practically no change in the fluorescence ratio. Whereas, thirdly, in *Chlorella vulgaris* C3, the quantum requirements of low- and high-CO$_2$ cells were the same, but the fluorescence ratio was higher in high-CO$_2$ cells than in low-CO$_2$ cells.

In the current contribution we extend our investigation to 3 cyanobacteria, known to require PS I activity for accumulation of DIC. In addition to quantum requirement and fluorescence emission at low temperature, we measured fluorescence induction in the presence of DCMU as indicator of PS II activity.

**Materials and Methods**

**Culture conditions**

Cells of *Anabaena variabilis* M3, *Anabaena variabilis* ATCC 29413 and *Anacystis nidulans* R2 (all obtained from the Algal Collection, Institute of Applied Microbiology, University of Tokyo) were grown photoautotrophically in culture-tubes (Bishop and Senger, 1971) at 28° C. The tubes were illuminated continuously with a bank of fluorescent lamps combination (Osram-L 40 W/15–1 / Osram-L 40 W/25–1) at intensities of 3 Wm$^{-2}$. Medium C of Kratz and Myers (1955) including HEPES-NaOH (20 mm, pH 7.8) was used as growth medium. Cell suspensions were continuously bubbled with air or air enriched with 4.4% CO$_2$ to obtain low- or high-CO$_2$ cells, respectively.

**Harvesting**

Cells were harvested by centrifugation and resuspended in HEPES-NaOH buffer (30 mm, pH 7.8) to a density of 50 μg chlorophyll per ml. For experiments in the absence of sodium, HEPES-KOH buffer was used. All glassware were rinsed twice with 13 n HNO$_3$. The cells were washed twice with Na-free buffer before the experiments.

**Quantum requirements**

Quantum requirement of photosynthetic oxygen evolution was measured with an integrating Ulbricht sphere containing a glass cuvette with an oxygen electrode (micro-Clark, Yellow-Springs Instr., Yellow Springs, Ohio, USA). This apparatus allowed the simultaneous measurements of oxygen evolution and light absorption by photosynthetic organisms (for details, see Bürger et al., 1988). Percent absorption was determined by measuring the photocurrent by the photo voltaic cells of the Ulbricht sphere during illumination of the sample in the reaction vessel. For calibration, black ink (100% absorption) and a suspension of extracted cells (0 % absorption) were used (Warburg and Krippahl, 1954; Senger, 1971).

The oxygen electrode was calibrated with air-saturated water and sodium dithionite (reducing agent) solution. For determination of the quantum requirement of photosynthetic oxygen evolution, the slope of oxygen production with increasing light intensity beyond the oxygen compensation point was followed. The measurements were performed twice each with 4 independently grown high-CO$_2$ cells and 6 independently grown low-CO$_2$ cells. In low-CO$_2$ cells of *A. variabilis* M3 and ATCC 29413, the results obtained from 2 cultures out of 6 were not considered because of unusual deviation. The experimental materials were directly obtained from cultures grown under low- or high-CO$_2$ conditions and measurements were carried out at 25° C. All samples absorbed between 92 % and 96 % of the actinic light.

**Low temperature fluorescence spectra**

Fluorescence emission spectra reflected from the sample surface in liquid nitrogen (77 K) were recorded with a Shimadzu spectrofluorometer RF 502 (Krupinska et al., 1985). The excitation wavelength was 570 nm. The emission slit width was 5 nm. The density of the fluorescence probes was 50 μg Chl·ml$^{-1}$. Dilution to half concentration did not alter the shape or wavelengths of the emission.
spectra. The samples were standardized with Rhodamin B to show changes in the height of the peaks in comparison to the chlorophyll content.

Fluorescence induction measurements

A cell suspension adjusted to a Chl-content of 5 μg ml⁻¹ was dark-adapted for 10 min. One minute after the addition of DCMU to a final concentration of 10⁻⁵ M, fluorescence induction was measured at room temperature with the photomultiplier of an Aminco DW-2 spectrophotometer (500 V voltage, Aminco, Silver Spring, USA) screened with an interference filter (half band width 8 nm, DIL 683, Schott, Mainz, Germany) with an intensity of 1 W m⁻². The curves were monitored with a storage oscilloscope (Tektronix 5A22N, differential amplifier and Tektronix 51312N oscilloscope, Tektronix, Beaverton, Oregon, USA) attached to the photomultiplier.

Results and Discussion

Changes in quantum requirement of photosynthetic oxygen evolution reflect differences in the efficiency of the photosynthetic electron transport chain and/or the energy distribution between PS II and PS I (Myers, 1963). Measurements of the wavelength dependent quantum yield of high-CO₂ grown cells of *Anabaena variabilis* M3 demonstrate that quantum yield is in its maximum state at around 680 nm (data not shown). This is known to be the wavelength region in cyanobacteria as well as in green algae for the excitation of both photosystem I and II (Duysens and Amesz, 1962). Thus all quantum requirement measurements reported here were carried out at 679 nm.

In the present experiments the changes in the slopes of the photosynthetic oxygen evolution in *Anabaena variabilis* M3, *A. variabilis* ATCC 29413 and *Anacystis nidulans* R2 indicated that the quantum requirement of photosynthetic oxygen evolution was higher in low-CO₂ cells than in high-CO₂ cells (Fig. 1). The values for quantum requirement in photosynthesis are shown in Table I. The lowest quantum requirements were found in high-CO₂ cells of the *Anabaena* strains with values around 8.5 quanta per one molecule of O₂. The highest quantum requirement was found in low-CO₂ cells of *Anacystis nidulans* with 15.8 quanta per molecule O₂. Low-CO₂ cells of the cyanobacteria absorbed almost 2-3 quanta more to produce one molecule O₂ than high-CO₂ cells.

Mörschel and Rhiel (1987) stated in their review that fluorescence emission spectra of cyanobacteria in liquid nitrogen (77 K) showed peaks (or shoulders) in the regions around 610 nm, 640 nm, 660 nm, due to phycoerythrocyanin, phycocyanin, and allophycocyanin, respectively. According to Murata (1968) cyanobacteria also show peaks or shoulders at around 685-695 nm and 730 nm, which are mainly emitted by the chlorophylls of PS II and PS I, respectively. That the emission

![Fig. 1. Light response curves of photosynthetic oxygen evolution at 679 nm in low- and high-CO₂ cells of *Anabaena variabilis* M3, *Anabaena variabilis* ATCC 29413, and *Anacystis nidulans* R2. Solid line and dotted line show the results with high-and low-CO₂ cells, respectively. Light-responses were followed beyond the oxygen compensation point and corrected for dark respiration. Mean values of 8–12 measurements with 4–6 different materials are used to draw the figure. Standard deviation of the middle area of the curves was at around ± 10%.](image-url)
Table I. Quantum requirements of photosynthetic oxygen evolution at 679 nm, ratio of $F_{710-740}$ to $F_{680-700}$ fluorescence at liquid nitrogen temperature (77 K), and relative fluorescence emission at the 680–700 nm and 710–740 nm maxima on the basis of the same chlorophyll content in low- and high-CO₂ cells of Anabaena variabilis M3, Anabaena variabilis ATCC 29413, and Anacystis nidulans R2. Fluorescence samples were standardized with Rhodamin B. Excitation wavelength 570 nm.

<table>
<thead>
<tr>
<th>Materials</th>
<th>Quantum requirement</th>
<th>Fluorescence /77 K</th>
<th>Excit. 570 nm</th>
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<tbody>
<tr>
<td></td>
<td>high CO₂</td>
<td>low CO₂</td>
<td>F-ratio($F_{710-740}/F_{680-700}$)</td>
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<tr>
<td>Anabaena variabilis M3</td>
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<td>10.8</td>
<td>1.4</td>
</tr>
<tr>
<td>A. variabilis ATCC 29413</td>
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<td>11.1</td>
<td>1.6</td>
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<tr>
<td>Anacystis nidulans R2</td>
<td>13.6</td>
<td>15.8</td>
<td>0.9</td>
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</table>

around 730 nm mainly arises from the light-collecting antenna of PS I was also shown by Butler and Kitajima (1975). A higher ratio of fluorescence at 715–740 nm to 685–695 nm indicates a higher transfer of excitation energy to PS I (Murata, 1969).

It was previously shown that the ratio of $F_{715-740}$ to $F_{685-695}$ in low-CO₂ cells were higher than in high-CO₂ cells of Dunaliella tertiolecta, Chlorella vulgaris 11g (Bürger et al., 1988), and Chlamydomonas reinhardtii (Bürger et al., 1988; Palmqvist et al., 1990). Since the quantum requirements of oxygen evolution were also higher in low-CO₂ cells than in high-CO₂ cells, it was assumed that the low-CO₂ cells of these unicellular green algae needed a higher amount of light acting on PS I than high-CO₂ cells. The same effects of CO₂ concentration on the fluorescence ratio were observed in the cyanobacteria investigated here (Fig. 2, Table I). Using Rhodamin B as a fluorescence standard, it was further shown that only the amount of the $F_{680-700}$ emission decreased in Anabaena variabilis ATCC 29413 and Anacystis nidulans R2 in association with lowering CO₂ level during growth. However, $F_{680-700}$ emission was lower, while $F_{710-740}$ emission was higher in low-CO₂ cells than in high-CO₂ cells of Anabaena variabilis M3.

The response of PS II activity upon changes in CO₂ concentration could be determined by measurement of the fluorescence induction kinetics in the presence of DCMU (Fig. 3, Table II). Photosynthetic organisms treated with DCMU in the dark typically exhibit a fast initial fluorescence $(F₀)$ after illumination. Then fluorescence rises to a constant maximum level $(F_{max})$ within the first 500 ms, due to the reduction of the electron acceptor Q of photosystem II. Comparison of different $F₀$ levels is difficult, since not only the fluorescence of PS II antenna chlorophylls, but also those of unconnected chlorophylls, and the short wavelength antenna chlorophyll of PS I are involved in these levels (Kitajima and Butler, 1975; Akoyunoglou, 1977; Krause and Weis, 1984). The half rise time $t_{1/2}$ of the fluorescence induction which represents one half of the time required to accomplish the reduction of Q in the presence of DCMU is proportional to the number of Q molecules (Dubertret and Joliot, 1974) and thus correlates with...
the amount of PS II reaction centers in the sample. Actually there was no significant difference in $t_{1/2}$ between low- and high-CO$_2$ cells (Table II). Thus we have to conclude that the size of PS II reaction centers of all 3 cyanobacteria is identical in low and high CO$_2$-adapted cells.

For evaluating the excitation of PS II, the variable fluorescence ($F_{\text{var}}=F_{\text{max}}-F_0$), which is the difference between the fluorescence of the oxidized PS II centers ($F_0$) and the reduced PS II centers ($F_{\text{max}}$), was chosen. A lower value of $F_{\text{var}}$ in the cells with the same chlorophyll content and excited with the same actinic light indicates a lower excitation of photosystem II. For the three cyanobacteria the variable fluorescence ($F_{\text{var}}$) was higher in high-CO$_2$ cells than in low-CO$_2$ cells, indicating a lower excitation of PS II in the latter cells (Fig. 3, Table II). These findings are in agreement with the data of the low-temperature fluorescence spectra. The initial fluorescence $F_0$ also was higher in high-CO$_2$ cells than in low-CO$_2$ cells.

The mechanism that causes the disproportion between the electron flow through the two photosystems in low CO$_2$ adapted cells is not known. Since the adaptational change from the one to the other condition takes about one day, it was discussed by Müller et al. (1994) that growth phenomena and protein biosynthesis might be involved. However, it can not be excluded at the current state of knowledge that a regulation in the electron transport chain between PS II and PS I takes place.

It should be mentioned that all measurements were also performed in parallel with algae grown in the absence of sodium in Na$^+$-free measuring buffer. Abe et al. (1987) showed that the light-dependent transport of inorganic carbon was suppressed in the absence of sodium in low-CO$_2$ cells of Anabaena variabilis M3. They assumed that sodium was required for the active transport of inorganic carbon during photosynthesis. However, no significant influence of sodium on the photosynthetic characteristics could be observed in the present experiments (data not shown).

The cyanobacteria Anabaena variabilis M3, A. variabilis ATCC 29413, and Anacystis nidulans R2 show a higher quantum requirement for photosynthetic oxygen evolution, and a higher ratio of $F_{710-740}$ to $F_{680-700}$ in the low temperature fluorescence emission in low-CO$_2$ cells compared to high-CO$_2$ cells. This type of response indicates that in low-CO$_2$ cells more light absorbed by PS I is used by a process other than the oxidation of the PS II electron acceptor via the linear electron transport chain. It is most probable that this energy is used for the cyclic electron flow of PS I to drive the inorganic carbon pump in low-CO$_2$ cells of Anabaena variabilis and Anacystis nidulans as suggested by Ogawa et al. (1984, 1985). The same photosynthetic characteristics were observed in the green
Table II. Fluorescence induction parameters in the presence of DCMU (10⁻⁵ m) of low- and high-CO₂ cells of Anabaena variabilis M3, Anabaena variabilis ATCC 29413, and Anacystis nidulans R2. Relative values.

<table>
<thead>
<tr>
<th>Materials</th>
<th>high CO₂</th>
<th>low CO₂</th>
<th>high CO₂</th>
<th>low CO₂</th>
<th>high CO₂</th>
<th>low CO₂</th>
<th>high CO₂</th>
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<td>8.7</td>
<td>6.7</td>
<td>4.1</td>
<td>2.5</td>
<td>2.0</td>
<td>2.2</td>
</tr>
<tr>
<td>A. variabilis ATCC 29413</td>
<td>5.0</td>
<td>3.6</td>
<td>8.9</td>
<td>5.0</td>
<td>3.9</td>
<td>1.4</td>
<td>1.9</td>
<td>1.9</td>
</tr>
<tr>
<td>Anacystis nidulans R2</td>
<td>4.9</td>
<td>3.7</td>
<td>7.2</td>
<td>4.4</td>
<td>2.3</td>
<td>1.3</td>
<td>3.0</td>
<td>3.2</td>
</tr>
</tbody>
</table>

alga Dunaliella tertiolecta, Chlamydomonas reinhardtii C-9, and Chlorella vulgaris 11g (Bürg er et al., 1988).

It was reported that Anacystis nidulans cells grown with 3 % CO₂ showed greater phycocyanin to chlorophyll ratio relative to cells grown with 0.2 % CO₂ (Eley, 1971). Manodori and Melis (1984) further showed that the photosystem II/photosystem I reaction center ratio was higher in high-CO₂ cells than in low-CO₂ cells of Anacystis nidulans.

Their results are in good accordance with our data.

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Kaplan A., Zenwirth D., Reinhold L. and Berry J.A. (1982), Involvement of a primary electrogenic pump in the mechanism for HCO₃⁻-uptake by the cyanobacterium Anabaena variabilis. Plant Physiol. 69, 978–982.


